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miR-149 rs2292832 C>T polymorphism and risk of gastric cancer

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Abstract

Accumulating evidence that microRNA (miRNA) genes are involved in different processes associated with gastric carcinogenesis. The polymorphisms located on miRNA sequences may affect the interaction with their target messenger RNAs (mRNAs) and, consequently, genetic susceptibility to disease. The aim of our study was to investigate the association of miR-149 rs2292832 C>T polymorphism and gastric cancer susceptibility in Romanian patients. A total of 142 patients with gastric adenocarcinoma and 288 healthy controls were included in this study. The miR-149 rs2292832 allelic variants were genotyped by real-time polymerase chain reaction (RT-PCR) using specific TaqMan predesigned probes. The association between polymorphism and gastric cancer risk was estimated by odds ratio (OR) and 95% confidence interval (CI). The miR-149 rs2292832 C>T was not associated with susceptibility to gastric cancer, when TT genotype was compared with the more frequent AA genotype (OR 0.98, 95% CI 0.55–1.77, p=0.96) or when we used dominant and recessive models. Also, we compared allele frequencies and no correlation was found (OR 0.92, 95% CI 0.68–1.24, p=0.57). The sub-classification of gastric cancer into non-cardia and cardia or intestinal and diffuse type did not reveal any statistically significant difference for investigated polymorphism.

Keywords: gastric cancer, miRNA, polymorphism, genotype, susceptibility.

Introduction

Gastric cancer (GC) is the third cause of cancer-related deaths in both sexes worldwide [1]. The overall prognosis for GC is poor and the 5-year survival rate for advanced GC is less than 20–30% [2, 3]. Gastric cancer is a multifactorial disease involving both environmental factors and host genetic susceptibility [4], and it is well known that *Helicobacter pylori* infection plays a critical role.

MicroRNAs (miRNAs) are a group of endogenously expressed, evolutionarily well-conserved and non-coding small RNAs, with a length of 18–25 ribonucleotides. MiRNAs recognize and complementarily directly binding with the 3'UTR (untranslated region) of the target messenger RNAs (mRNAs), thereby negatively regulate gene expression at the post-transcriptional level by suppressing translation, mRNA cleavage or decreasing the stability of mRNAs [5, 6]. Accumulating evidence indicates that miRNAs play important roles in development, metabolism, proliferation, differentiation, apoptosis, angiogenesis and immune response, processes, which are known to have essential roles in carcinogenesis [7–9]. Moreover, some miRNAs can function as either oncogenes or tumor suppressors by regulating the expression of different target genes involved in carcinogenesis [9]. More specifically, miRNAs negatively regulate the expression of cancer-related genes by enhancing the expression of oncogenes or decreasing the expression of tumor suppressor genes [10]. A single miRNA molecule can target hundreds to thousands of mRNAs [11, 12] and about 50% miRNA genes are located at cancer-associated genomic regions [13, 14]. Mutations or single-nucleotide polymorphisms (SNPs) located at miRNA genes may affect the pre-miRNA maturation or the binding to target mRNAs, [15, 16]. Thus, minor variations in miRNA genes may alter multiple biological pathways [17], influencing interaction between miRNAs and their target mRNAs, and further host genetic susceptibility to various diseases, including cancer. It has been hypothesized that SNPs located in miRNA genes are promising biomarkers for digestive cancers, including GC [18, 19]. In addition, different miRNAs have been shown to participate in gastric cancer initiation, progression, pathways, and resistance to chemotherapy [20]. A large number of studies have been done to assess the association of numerous miRNA SNPs with gastric cancer risk, the miR-149 rs2292832 SNP being one of the most extensively investigated [18, 19]. The associations of these variants with GC were mainly conducted in Asian population with controversial results. Despite the high incidence and mortality by GC in Eastern Europe, the number of miRNA SNPs studies in Caucasian population is poor.

The purpose of our study was therefore to investigate the association of miR-149 rs2292832 C>T SNP and GC susceptibility in Romanian patients, an ethnic group in which the association between GC and this SNP has not previously been studied.

Subjects, Materials and Methods

Subjects

In this hospital-based case-control study, we included 142 GC patients and 288 healthy controls, recruited from the Emergency County Hospital of Craiova (Romania). Each GC patient underwent upper endoscopy and testing for the presence of *H. pylori*. The diagnoses of GC were all confirmed by histological examination of specimens obtained by endoscopic biopsy or after surgery. GC was histologically grouped according to the Lauren's classification as either intestinal or diffuse. Unrelated cancer-free controls matched to GC cases by gender and age, were selected from patients hospitalized. Individuals with any digestive diseases and tumors were excluded as controls. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova and informed consent was obtained from each participant.

TaqMan genotyping assay

Peripheral blood samples were taken from all patients and controls, and stored in EDTA (ethylenediaminetetraacetic acid) tubes. Genomic DNA was extracted from the peripheral blood leukocytes using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI), following the manufacturer protocol. The miR-149 rs2292832 C>T SNP was genotyped by real-time polymerase chain reaction (RT-PCR) using specific TaqMan predesigned probes (assay C__11533078_1, Applied Biosystems, Foster City, CA, USA). RT-PCR was performed on a ViiA™ 7 RT-PCR System (Life Technologies, Carlsbad, USA) and SNP genotyping was carried out in a 10 μL reaction volume.

To control the quality of genotyping, the TaqMan assay was performed without knowing the status of the cases or controls. In addition, 10% of the samples were randomly selected and retested using another RT-PCR system (RotorGene 6200 HRM-Corbett) in a 5 μL reaction volume as we previously described [21] and the results were 100% concordant.

Statistical analysis

Hardy–Weinberg equilibrium of the SNP genotypes was analyzed by the goodness-of-fit chi-square (χ^2) test. Odds ratio (OR) and 95% confidence interval (CI) were calculated to evaluate the association between the SNP and the risk of GC. χ^2 test was used to assess the difference in the distribution of genotype frequencies between cases and controls. We evaluated the risk under all contrast models: the codominant model (CC *vs.* CT & CC *vs.* TT) the dominant model (CC *vs.* CT+TT), the recessive model (CC+CT *vs.* TT), and the allelic model (C *vs.* T); all models assuming T is the risk allele.

Results

The characteristics of the subjects in the case and control groups are summarized in Table 1. The average age was 66.26 years and 62.6% of GC patients were men. In the control group, the average age was 64.49 years and 60.41% were men. Cases and controls were well matched in terms of gender and age, and no significant differences in gender and age were observed between the patients and controls. The number of patients with cancer of the gastric cardia and non-cardia was 31 and 111, respectively. All included cases were *H. pylori*-positive gastric adenocarcinoma and histopathological analysis revealed GC intestinal type in 79 cases, diffuse type in 62 cases and one was mixed type. Among controls, genotype distributions for assessed SNP was in Hardy–Weinberg equilibrium (χ^2=2.29, p=0.13).

The genotypes were identified according to FAM and VIC dyes (Figures 1–3).

Table 2 presents the frequencies of each genotype and the association between miR-149 rs2292832 C>T SNP and the risk of GC.

Table 1 – ***Subjects characteristics***

	Gastric adenocarcinoma (*n*=142)	Control (*n*=288)
Males/females ratio	89/53	174/114
Age [years], mean±SD (standard deviation)	66.26±8.65	64.49±7.94
Location:		
▪ *cardia*	31	
▪ *non-cardia*	111	
Histological type:		
▪ *intestinal*	79	
▪ *diffuse*	62	
▪ *mixed*	1	

Table 2 – ***Genotyping frequencies for miR-149 rs2292832 C>T SNP in cases and controls and the association with risk of overall GC***

Polymorphism	Gastric cancer (*n*=142)	Control (*n*=288)	OR (95% CI)	*P* value
miR-149 rs2292832				
CC	70 (49.3%)	126 (43.75%)	Reference	–
CT	49 (34.5%)	120 (41.67%)	0.74 (0.47–1.14)	0.17
TT	23 (16.2%)	42 (14.58%)	0.98 (0.55–1.77)	0.96
CC vs. CT+TT	72 (50.7%)	162 (56.25%)	0.8 (0.54–1.19)	0.28
CC+CT vs. TT	119 (83.8%)	246 (85.42%)	0.88 (0.51–1.54)	0.66
C	189 (66.55%)	372 (64.58%)	Reference	–
T	95 (33.45%)	204 (35.42%)	0.92 (0.68–1.24)	0.57

OR: Odds ratio; CI: Confidence interval.

No statistically significant difference was observed between GC patients and controls when we compared one genotype with other genotype (codominant models – the most common genotype serves as reference) or when we used dominant and recessive models. Also, we compared allele frequencies and no correlation was found (OR 0.92, 95% CI: 0.68–1.24, p=0.57). Moreover, association of this SNP with tumor site and histological type was examined separately and the sub-classification of GC into non-cardia and cardia or intestinal and diffuse did not reveal any statistically significant difference (Tables 3 and 4).

Table 3 – ***Risk of cardia and non-cardia adenocarcinoma by miR-149 rs2292832 genotype***

Polymorphism	Non-cardia (*n*=111)		Cardia (*n*=31)	
	n (%)	*OR (95% CI); p value*	*n (%)*	*OR (95% CI); p value*
miR-149 rs2292832				
CC	57 (51.35%)	Reference	13 (41.93%)	Reference
CT	37 (33.33%)	0.68 (0.42–1.11); 0.12	12 (38.71%)	0.97 (0.42–2.21); 0.94
TT	17 (15.32%)	0.89 (0.47–1.7); 0.74	6 (19.36%)	1.38 (0.49–3.87); 0.53
CC vs. CT+TT	54 (48.65%)	0.74 (0.48–1.14); 0.17	18 (58.07%)	1.08 (0.51–2.28); 0.85
CC+CT vs. TT	94 (84.68%)	0.94 (0.51–1.74); 0.85	25 (80.64%)	0.71 (0.28–1.84); 0.48
C	151 (68.02%)	Reference	38 (61.29%)	Reference
T	71 (31.98%)	0.86 (0.62–1.19); 0.36	24 (38.71%)	1.15 (0.67–1.97); 0.61

OR: Odds ratio; CI: Confidence interval.

Table 4 – ***Risk of intestinal and diffuse gastric adenocarcinoma by miR-149 rs2292832 genotype***

Polymorphism	Intestinal (*n*=79)		Diffuse (*n*=62)	
	n (%)	*OR (95% CI); p value*	*n (%)*	*OR (95% CI); p value*
miR-149 rs2292832				
CC	43 (54.43%)	Reference	26 (41.93%)	Reference
CT	27 (34.18%)	0.66 (0.38–1.13); 0.13	22 (35.49%)	0.89 (0.48–1.65); 0.71
TT	9 (11.39%)	0.63 (0.28–1.39); 0.25	14 (22.58%)	1.61 (0.77–3.38); 0.2
CC vs. CT+TT	36 (45.57%)	0.65 (0.39–1.07); 0.09	36 (58.07%)	1.08 (0.62–1.88); 0.79
CC+CT vs. TT	70 (88.61%)	1.33 (0.62–2.86); 0.47	48 (77.42%)	0.59 (0.29–1.15); 0.12
C	113 (71.52%)	Reference	74 (59.68%)	Reference
T	45 (28.48%)	0.73 (0.49–1.07); 0.1	50 (40.32%)	1.23 (0.83–1.83); 0.3

OR: Odds ratio; CI: Confidence interval.

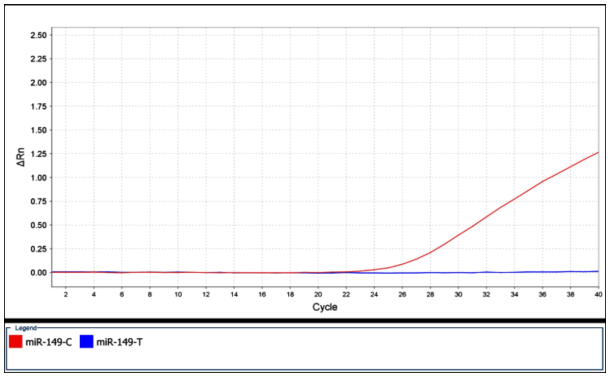

Figure 1 – ***Allelic discrimination according to FAM and VIC – CC genotype.***

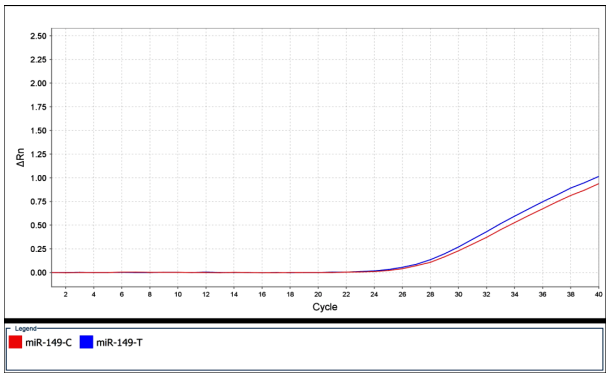

Figure 2 – ***Allelic discrimination according to FAM and VIC – CT genotype.***

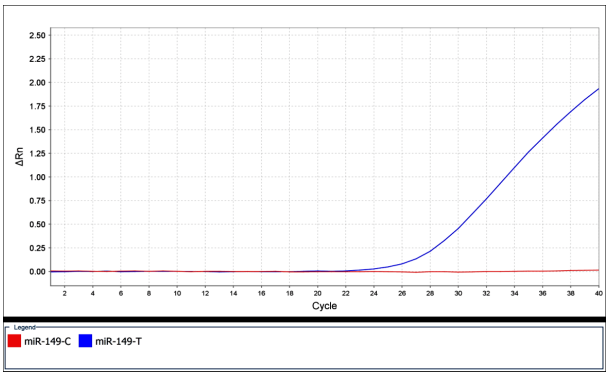

Figure 3 – ***Allelic discrimination according to FAM and VIC – TT genotype.***

Discussion

To our knowledge, this is the first study that assessed miR-149 rs2292832 C>T SNP and GC risk in an Eastern European population (Romanian population) and the second in a Caucasian population. We failed to find a correlation between miR-149 rs2292832 and GC susceptibility in any contrast model or in the stratified analysis by cancer site or histological type. Previous studies have investigated the association between the miR-149 rs2292832 C>T SNP and risk of cancer, including GC, with inconsistent and controversial results. Our findings are similar to several meta-analyses, where no significant associations were detected between this SNP and GC risk, containing mainly Asian populations [18, 22–24]. A significant association between miR-149 rs2292832 SNP and digestive cancer was identified in a meta-analysis, the carriers of CT genotype may have a decreased risk for digestive cancer than TT genotype, but no relationship was found for GC in the stratified analysis by type of cancer [23]. miR-149 rs2292832 was not correlated with cancer risk overall or in a subgroup analysis by ethnicity (Asian and Caucasian), study design (hospital-based and population-based) or cancer type, including GC [24]. No significant association was found between miR-149 SNP and GC risk in a case-control study conducted in the Chinese population [25]. Another meta-analysis did not reveal any association between the miR-149 rs2292832 SNP and cancer susceptibility [26], and no positive results were found in other two Asian populations [27, 28].

In contrast, Zhang *et al.* (2012) reported that smokers bearing the pre-miR-149 rs2292832 both CT and CC

genotypes had an increased susceptibility to gastric cancer whereas tea drinkers with the pre-miR-149 rs2292832 CT/CC genotypes were protected against gastric cancer [29]. The same study reported that male subjects with the pre-miR-149 rs2292832 CT genotype had a decreased susceptibility for gastric cancer in an Asian population. The carriers of heterozygous CT miR-149 rs2292832 genotype were associated with a decreased risk for gastric cancer when compared with the wild type in a recent meta-analysis [18]. Another meta-analysis reported that C allele is associated with protective role in overall cancer survival, but only a single GC study conducted in an Asian population was included [30]. Also, a decreased risk of C allele and CT genotype was observed in hepatocellular carcinoma [31]. In contrast, the only European study on miR-149 rs2292832 conducted on a Greek population revealed a higher risk for carriers of CC genotype to develop GC [32]. This association was observed under a homozygote comparison, dominant and recessive genetic models. In addition, miR-149 rs2292832 was associated with a slightly increased overall risk of gastrointestinal cancer risk in the recessive model, especially for Asians [33].

The minor allele frequency in our study (T allele – 35.42%) is comparable to that of Europeans published in the NCBI SNPdb (https://www.ncbi.nlm.nih.gov/projects/SNP), and similar to that reported in Caucasian population (25.2–36%) and significantly lower than the corresponding frequency in Asian population (53.2–77%) [22].

Our study has some limitations that should be mentioned. Firstly, all subjects were selected from only one hospital and the selection bias cannot be ruled out. GC susceptibility is influenced by multiple gene–gene interactions, which were not evaluated and more data about biological effects of the investigated SNP are required. Furthermore, the influence of T allele in miR-149 SNP might be masked by the presence of other uninvestigated genes and the sample size, which is relatively small.

Conclusions

miR-149 rs292832 C>T polymorphism is not associated with risk of gastric cancer in the Romanian population. More extensive studies are needed on different ethnic groups in order to improve the level of knowledge of the miRNA polymorphisms in gastric carcinogenesis.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Raluca Alexandra Cîmpeanu and Dragoş-Marian Popescu equally contributed to the manuscript and share main authorship.

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